Results: Results showed that treatment with AVE 0991 could reduce AngII-induced proliferation of cardiac fibroblasts, manifesting decreased DNA synthesis and collagen production. Moreover, treatment with AVE 0991 could attenuate AngII-induced activation of Smad signaling pathways. Furthermore, the beneficial effects and Smad pathways's changes of AVE0991 were abolished by A-779.
Conclusions: Our data showed that AVE 0991 attenuated proliferation of cardiac fibroblast, which may be due to the inhibition of Smad pathways.

GW25-e1651

Identification of Stim1 as a Candidate Gene for Exaggerated Sympathetic Response to Stress in the Stroke-Prone Spontaneously Hypertensive Rat

Xiao Bing[1,2], Liu Fan[1], Ohara Hiroki[2], Nabika Toru[2]
[1]Department of Cardiology, The Second Hospital of Hebei Medical University, Shijiazhuang, Hebei province, China, [2]Department of Functional Pathology, Shimane University School of Medicine, Izumo, Japan

Objectives: The stroke-prone spontaneously hypertensive rat (SHRSP) is known to have exaggerated sympathetic nerve activity to various types of stress, which might contribute to the pathogenesis of severe hypertension and stroke observed in this strain. Previously, by using a congenic strain (called SPwch1.72) constructed between SHRSP and the normotensive Wistar-Kyoto rat (WKY), we showed that a 1.8-Mbp fragment on chromosome 1 (Chr1) of SHRSP harbored the responsible gene (s) for the exaggerated sympathetic response to stress. To further narrow down the candidate region, in this study, another congenic strain (SPwch1.71) harboring a smaller fragment on Chr1 including two functional candidate genes, Phox2a and Ship2, was generated.
Methods: Sympathetic response to cold and restraint stress was compared among SHRSP, SPwch1.71, SPwch1.72 and WKY by three different methods (urinary norepinephrine excretion, blood pressure measurement by the telemetry system and the power spectral analysis on heart rate variability).
Results: The results indicated that the response in SPwch1.71 did not significantly differ from that in SHRSP, excluding Phox2a and Ship2 from the candidate genes. As the stress response in SPwch1.72 was significantly less than that in SHRSP, it was concluded that the 1.2-Mbp congenic region covered by SPwch1.72 (and not by SPwch1.71) was responsible for the sympathetic stress response. The sequence analysis of 12 potential candidate genes in this region in WKY/Izm and SHRSP/Izm identified a nonsense mutation in the stromal interaction molecule 1 (Stim1) gene of SHRSP/Izm which was shared among 4 substrains of SHRSP. A western blot analysis confirmed a truncated form of STIM1 in SHRSP/Izm. In addition, the analysis revealed that the protein level of STIM1 in the brainstem of SHRSP/Izm was significantly lower when compared with WKY/Izm.
Conclusions: Our results suggested that Stim1 is a strong candidate gene responsible for the exaggerated sympathetic response to stress in SHRSP.

GW25-e1653

SIRT1 enhances Therapeutic Efficacy of Aged Mesenchymal Stem Cells in Rat Myocardial Infarction via lightening MSCs Aging and heightening Stress Resistance

Huiqiang Chen, Jian-an Wang
Cardiovascular Key Lab of Zhejiang Province, the Second Affiliated Hospital, School of Medicine, Zhejiang University

Objectives: Mesenchymal stem cells (MSCs) hold great promise for stem cell therapy and are gradually used in clinical trials in recent years. However, advanced age is a negative factor for autologous MSCs transplantation.
Methods: For in vitro study, we evaluated cell senescence using SA-B-gal method while for evalulated the stress response we conducted H2O2-induced hypoxia/ischemia model in MSCs. The conditioned medium was collected and was added to HUVEC to observe the alteration of tube-formation between vectot-aged MSCs and SIRT1-aged MSCs. For experiments in vivo, we first conducted ischemic female heart model induced by left anterior descending (LAD) ligation. At day 3 after male cell transplantation, the cell survival was evaluated by SRY detection. At day 28 after cell transplantation, heart function and fibrosis were analyzed by ECHO and Masson staining respectively. Morover, vascular density was evaluatied by CD31 and SMA staining.
Results: In the present study, we evaluated the effect of SIRT1 overexpression in MSCs from aged rat in vivo and in vitro. Compared with vector-aged MSCs, SIRT1 modified-aged MSCs significantly promote cellular survival in the ischemic heart induced by left anterior descending ligation at day 3 after cell transplantation. Moreover, SIRT1 overexpression in aged MSCs could down-regulate fibrosis and up-regulate vascular density, consequently contribute to the improvement of heart function. In vitro data demonstrated that forced expression of SIRT1 inaged MSCs could contribute to significantly attenuation of MSCs senescence and the augmentation of MSCs survival under hypoxia/ischemia condition. The improvement of angiogenesis was also observed in vitro when SIRT1 was overexpressed in aged MSCs compared with vector-aged MSCs, which might be attributed to the up-regulation of paracrine factors including AngI and bFGF.
Conclusions: Together, our data support the notion that forced expression of SIRT1 inaged MSCs could ameliorate the phenotypes and function of aged MSCs, consequently contribute to the improvement of cell-based therapy for myocardial infarction.

GW25-e2290

Effect of Sini Decoction on the expression of SOD and MDA in EAhy926 cells injured by homocysteine

Liu Yong, Song Zhiming, Zhou Bin, Yu Shujie, Liu Dinghui, Hao Baoshun, Wu Lin, Wang Min, Chen Lin, Qian Xiaoxian
Department of Cardiology, The Third Affiliated Hospital of Sun Yat-sen University

Objectives: To detect the effect of Sini Decoction on the expression of SOD and MDA in EAhy926 cell injured by homocysteine.
Methods: Model of EAhy926 cell injured by homocysteine was made, the protection on the EAhy926 cell of Sini Decoction with different dosages were observed. SOD and MDA concentration of cell culture fluid was detected, effect of Sini Decoction on the expression of protein of SOD1 in EAhy926 cells were observed by Western-blot, and effect of Sini Decoction on the expression of mRNA of SOD1 in EAhy926 cell were observed by fluorescent quantitation PCR.
Results: After Model of EAhy926 cell injured by homocysteine was made, we found that cultured with 0.5, 1.0, 2.0, 4.0, 8.0μmol/L homocysteine, cells grew less than cultured with normal culture medium, as culturing with homocysteine 4.0μmol/L for 24h did lower damage to cells and could induce effective cell injuring, it was made to be the model of injury. To detect the effect of Sini Decoction on EAhy926 cell injured by homocysteine, well growing EAhy926 cells were cultured in culture plate. 24h later, cells were cultured with medium containing Sini Decoction 0, 0.25, 0.5, 1.0g/ml respectively for 30 minutes, then cultured with medium containing homocysteine 4.0μmol/L for 24h. It was found that, compared with control group, attached cells in Sini Decoction groups grew better, and attached cells in Sini Decoction 1.0g/ml plus homocysteine 4.0μmol/L group grew best. It was found that compared with control group, there was no obvious change of SOD (5.7765±0.1602 vs 5.6087±0.661u/ml) and MDA (0.6667±0.1649 vs 0.6775±0.1528 nmol/ml) concentration of cell culture fluid in Sini Decoction 1.0 g/ml group, but in homocysteine 4.0μmol/L medol group, SOD (2.6434±0.5765 u/ml) concentration of cell culture fluid decreased oberviously and MDA (1.2381±0.1649 nmol/ml) concentration of cell culture fluid increased oberviously, while in Sini Decoction groups, SOD concentration of cell culture fluid increased and MDA concentration of cell culture fluid decreased, in Sini Decoction 1.0g/ml plus homocysteine 4.0μmol/L group it was the most obvious (SOD4.9599±0.5884 u/ml, MDA0.7143±0.1428 nmol/ml, $P<0.05$). Detected by Western-blot, it was found that, campared with control group, there was no obervious change of protein of SOD1 in Sini Decoction 1.0 g/ml group, but in homocysteine 4.0μmol/L model group, expression of SOD1 protein weakened obviously, and in Sini Decoction groups, expression of SOD1 protein enhanced, and in Sini Decoction 1.0g/ml plus homocysteine 4.0μmol/L group it was the most obvious ($P<0.05$). Detected by fluorescent quantitation, it was found that, compared with control group, there was no obvious change of mRNA of SOD1 in Sini Decoction 1.0g/ml group, but in homocysteine 4.0μmol/L model group, expression of SOD1 mRNA weakened obviously, and in Sini Decoction groups, expression of SOD1 mRNA enhanced, and in Sini Decoction 1.0g/ml Plus homocysteine 4.0μmol/L group, it was the most obvious ($P<0.05$).
Conclusions: Homocysteine may injure EAhy926 cell by suppressing the expression of SOD, while Sini Decoction may protect EAhy926 cell by enhancing the expression of SOD.

GW25-e2345

MiR-211 mediated hypoxia induce bone marrow mesenchymal stem cells migration through STAT5A

Hu Xinyang, Jian-an Wang
Cardiovascular Key Lab of Zhejiang Province, the Second Affiliated Hospital, School of Medicine, Zhejiang University

Objectives: Efficacy of intravenous administration of mesenchymal stem cells (MSCs) for myocardial infarction (MI) is limited by low cell migration to the damaged myocardium. Our previous study demostrated that migration ability of MSCs enhanced by hypoxia preconditioning (HPC). miRNA microarray displayed that miR-211 exhibited the most significant change between HPC and normoxia cultured MSCs. The aim of this study is to identify whether miR-211 regulates MSCs migration and the potential mechanism.
Methods: In vitro, transwell assay were used to assess the migration ability of MSC moduated by miR-211 using miR-211 overexpressing and knockdown lentivirus. The target gene of miR-211 predicted by Targetscan, which were verified by PCR, western blot and lucifurase assay. We knocked down the target gene and assessed the migration capability again. Chromatin immunoprecitation (ChiP) were used to explore the transcription factors that regulate the expression of miR-211. To evaluate the effect of miR-211 on MSCs migration in vivo, miR-211-mimic and miR-211-shRNA MSCs from male mice were intravenously delivered 24h after MI, the engraft cells were detected by RT-PCR of SRY gene.
Results: Quatitative RT-PCR showed that miR-211 expression of MSCs upregulated by HPC. MiR-211 mimic improved MSCs migration by 31.03% ($P<0.05$), however, knockdown miR-211 using shRNA attenuated MSCs migration ability significantly. Signal transducer and activator of transcription 5A (STAT5A) was predicted as one of target genes of miR-211, and was confirmed by PCR and Western blot, it showed miR-211 overexpression dramatically decreased STAT5A expression, while miR-211 knockdown upregulated STAT5A. The luciferase assay showed the similar results. Functional experiment showed that STAT5A knockdown reverse the inhibition of MSCs migration induced by miR-211-shRNA. Intrestingly, ChiP assay showed that